

This listing of claims will replace all prior versions, and listings, of claims in the application:

**Listing of Claims:**

1. **(Withdrawn)** A pair of fusion proteins consisting of a first fusion protein comprising segments P1, Cub-X, and RM, in an order wherein Cub-X is closer to the N-terminus of the first fusion protein than RM, and a second fusion protein comprising segments Nux and P2, wherein:

P1 or P2 or both is a membrane-associated protein, and P2 may be the same or different from P1;

Nux is the amino-terminal subdomain of a wild-type ubiquitin or a reduced-associating mutant ubiquitin amino-terminal subdomain;

Cub is the carboxy-terminal subdomain of a wild-type ubiquitin;

X is an amino acid other than methionine;

RM is an reporter moiety, and,

wherein the binding that occurs between P1 and P2 results in reassociation of Nux and Cub, thereby permitting ubiquitin-specific protease cleavage between Cub and X.

2. **(Withdrawn)** A pair of fusion proteins consisting of a first fusion protein comprising segments P1, Cub-X, and RM, in an order wherein Cub-X is closer to the N-terminus of the first fusion protein than RM, and a second fusion protein comprising segments Nux and P2, wherein:

P1 or P2 or both is a transcription factor;

Nux is the amino-terminal subdomain of a wild-type ubiquitin or a reduced-associating mutant ubiquitin amino-terminal subdomain;

Cub is the carboxy-terminal subdomain of a wild-type ubiquitin;

X is an amino acid other than methionine;

RM is an reporter moiety, and,

wherein the binding that occurs between P1 and P2 results in reassociation of Nux and Cub, thereby permitting ubiquitin-specific protease cleavage between Cub and X.

3. **(Withdrawn)** A pair of fusion proteins consisting of a first fusion protein comprising segments P1, Cub-X, and RM, in an order wherein Cub-X is closer to the N-terminus of the first fusion protein than RM, and a second fusion protein comprising segments Nux and P2, wherein:

P1 or P2 or both is a membrane-associated protein, and P2 may be the same or different from P1;

Nux is the amino-terminal subdomain of a wild-type ubiquitin or a reduced-associating mutant ubiquitin amino-terminal subdomain;

Cub is the carboxy-terminal subdomain of a wild-type ubiquitin;

X is an amino acid;

RM is an enzymatically active reporter moiety, and,

wherein the binding that occurs between P1 and P2 results in reassociation of Nux and Cub, thereby permitting ubiquitin-specific protease cleavage between Cub and X.

4. **(Withdrawn)** A pair of fusion proteins consisting of a first fusion protein comprising segments P1, Cub-X, and RM, in an order wherein Cub-X is closer to the N-terminus of the first fusion protein than RM, and a second fusion protein comprising segments Nux and P2, wherein:

P1 or P2 or both is transcription factor;

Nux is the amino-terminal subdomain of a wild-type ubiquitin or a reduced-associating mutant ubiquitin amino-terminal subdomain;

Cub is the carboxy-terminal subdomain of a wild-type ubiquitin;

X is an amino acid;

RM is an enzymatically active reporter moiety, and,

wherein the binding that occurs between P1 and P2 results in reassociation of Nux and Cub, thereby permitting ubiquitin-specific protease cleavage between Cub and X.

5. **(Withdrawn)** The pair of fusion proteins of claim 1, 2, 3, or 4, wherein X is Arginine.

6. **(Withdrawn)** The pair of fusion proteins of claim 1, 2, 3, or 4, wherein X is selected from the group consisting of Lysine, Histidine, Phenylalanine, Tryptophan, Tyrosine, Leucine, Aspartate, Glutamate, Cysteine, Asparagine, Glutamine and Isoleucine.
7. **(Withdrawn)** The pair of fusion proteins of claim 3 or 4, wherein X is Methionine, Glycine or Valine.
8. **(Withdrawn)** The pair of fusion proteins of claim 1, 2, 3, or 4, wherein the reporter moiety is a selectable marker.
9. **(Withdrawn)** The pair of fusion proteins of claim 8, wherein the selectable marker is selected from the group consisting of: URA3, HIS3, LYS2, HygTk, Tkneo, TkBSD, PACTk, HygCoda, Codaneo, CodaBSD, PACCoda, Tk, codA, GPT2, and HPRT.
10. **(Withdrawn)** The pair of fusion proteins of claim 8, wherein the selectable marker is selected from the group consisting of: TRP1, CYH2, CAN1.
11. **(Withdrawn)** The pair of fusion proteins of claim 1 or 2, wherein the reporter moiety is selected from the group consisting of: a transcription factor and a fluorescent marker.
12. **(Withdrawn)** The pair of fusion proteins of claim 1, 2, 3, or 4, wherein Nux contains at least one point mutation at amino acid 3 or amino acid 13 of a ubiquitin.
13. **(Withdrawn)** One or more nucleic acids that encodes or that together encode a first fusion protein comprising segments P1, Cub-X, and RM, in an order wherein Cub-X is closer to the N-terminus of the first fusion protein than RM, and a second fusion protein comprising segments Nux and P2, wherein:
  - P1 or P2 or both is a membrane-associated protein, and P2 may be the same or different from P1;
  - Nux is the amino-terminal subdomain of a wild-type ubiquitin or a reduced-associating mutant ubiquitin amino-terminal subdomain;
  - Cub is the carboxy-terminal subdomain of a wild-type ubiquitin;
  - X is an amino acid other than methionine;
  - RM is a reporter moiety, and,

wherein the binding that occurs between P1 and P2 results in reassociation of Nux and Cub, thereby permitting ubiquitin-specific protease cleavage between Cub and X.

14. **(Withdrawn)** One or more nucleic acids that encodes or that together encode a first fusion protein comprising segments P1, Cub-X, and RM, in an order wherein Cub-X is closer to the N-terminus of the first fusion protein than RM, and a second fusion protein comprising segments Nux and P2, wherein:

P1 or P2 or both is a membrane-associated protein, and P2 may be the same or different from P1;

Nux is the amino-terminal subdomain of a wild-type ubiquitin or a reduced-associating mutant ubiquitin amino-terminal subdomain;

Cub is the carboxy-terminal subdomain of a wild-type ubiquitin;

X is an amino acid;

RM is an enzymatically active reporter moiety, and,

wherein the binding that occurs between P1 and P2 results in reassociation of Nux and Cub, thereby permitting ubiquitin-specific protease cleavage between Cub and X.

15. **(Withdrawn)** One or more nucleic acids that encodes or that together encode a first fusion protein comprising segments P1, Cub-X, and RM, in an order wherein Cub-X is closer to the N-terminus of the first fusion protein than RM, and a second fusion protein comprising segments Nux and P2, wherein:

P1 or P2 or both is a transcription factor;

Nux is the amino-terminal subdomain of a wild-type ubiquitin or a reduced-associating mutant ubiquitin amino-terminal subdomain;

Cub is the carboxy-terminal subdomain of a wild-type ubiquitin;

X is an amino acid other than methionine;

RM is a reporter moiety, and,

wherein the binding that occurs between P1 and P2 results in reassociation of Nux and Cub, thereby permitting ubiquitin-specific protease cleavage between Cub and X.

16. **(Withdrawn)** One or more nucleic acids that encodes or that together encode a first fusion protein comprising segments P1, Cub-X, and RM, in an order wherein Cub-X is closer to the N-terminus of the first fusion protein than RM, and a second fusion protein comprising segments Nux and P2, wherein:

P1 or P2 or both is a transcription factor;

Nux is the amino-terminal subdomain of a wild-type ubiquitin or a reduced-associating mutant ubiquitin amino-terminal subdomain;

Cub is the carboxy-terminal subdomain of a wild-type ubiquitin;

X is an amino acid;

RM is an enzymatically active reporter moiety, and,

wherein the binding that occurs between P1 and P2 results in reassociation of Nux and Cub, thereby permitting ubiquitin-specific protease cleavage between Cub and X.

17. **(Withdrawn)** A method of determining whether two proteins, at least one of which is a membrane-associated protein, bind to each other comprising the steps of :

translationally providing a first fusion protein comprising segments P1, Cub-X, and RM, in an order wherein Cub-X is closer to the N-terminus of the first fusion protein than RM, and a second fusion protein comprising segments Nux and P2, wherein P1 and P2 are proteins, at least one of which is membrane-associated, which proteins may be the same or different, Nux is the amino-terminal subdomain of a wild-type ubiquitin or a reduced-associating mutant ubiquitin amino-terminal subdomain, Cub is the carboxy-terminal subdomain of a wild-type ubiquitin, X is an amino acid other than methionine and RM is an active reporter moiety; and

detecting the degree of cleavage by a ubiquitin-specific protease of the first fusion protein between Cub and X by detecting the degree of the activity of RM, wherein an increase of cleavage is indicative of P1/P2 binding.

18. **(Withdrawn)** A method of determining whether two proteins, at least one of which is a transcription factor, bind to each other comprising the steps of :

translationally providing a first fusion protein comprising segments P1, Cub-X, and RM, in an order wherein Cub-X is closer to the N-terminus of the first fusion protein

than RM, and a second fusion protein comprising segments Nux and P2, wherein P1 and P2 are proteins, at least one of which is a transcription factor, Nux is the amino-terminal subdomain of a wild-type ubiquitin or a reduced-associating mutant ubiquitin amino-terminal subdomain, Cub is the carboxy-terminal subdomain of a wild-type ubiquitin, X is an amino acid other than methionine and RM is an active reporter moiety; and

detecting the degree of cleavage by a ubiquitin-specific protease of the first fusion protein between Cub and X by detecting the degree of the activity of RM, wherein an increase of cleavage is indicative of P1/P2 binding.

19. **(Withdrawn)** A method of determining whether two proteins bind to each other, at least one of which is a membrane-associated protein, comprising the steps of :

translationally providing a first fusion protein comprising segments P1, Cub-X, and RM, in an order wherein Cub-X is closer to the N-terminus of the first fusion protein than RM, and a second fusion protein comprising segments Nux and P2, wherein P1 and P2 are proteins, at least one of which is membrane-associated, which proteins may be the same or different, Nux is the amino-terminal subdomain of a wild-type ubiquitin or a reduced-associating mutant ubiquitin amino-terminal subdomain, Cub is the carboxy-terminal subdomain of a wild-type ubiquitin, X is an amino acid and RM is an enzymatically active reporter moiety; and

detecting the degree of cleavage by a ubiquitin-specific protease of the first fusion protein between Cub and X by detecting the degree of the enzymatic activity of RM, wherein an increase of cleavage is indicative of P1/P2 binding.

20. **(Withdrawn)** A method of determining whether two proteins bind to each other, at least one of which is a transcription factor, comprising the steps of :

translationally providing a first fusion protein comprising segments P1, Cub-X, and RM, in an order wherein Cub-X is closer to the N-terminus of the first fusion protein than RM, and a second fusion protein comprising segments Nux and P2, wherein P1 and P2 are proteins, at least one of which is a transcription factor, Nux is the amino-terminal subdomain of a wild-type ubiquitin or a reduced-associating mutant ubiquitin amino-terminal subdomain, Cub is the carboxy-terminal subdomain of a

wild-type ubiquitin, X is an amino acid and RM is an enzymatically active reporter moiety; and,

detecting the degree of cleavage by a ubiquitin-specific protease of the first fusion protein between Cub and X by detecting the degree of the enzymatic activity of RM, wherein an increase of cleavage is indicative of P1/P2 binding.

21. **(Withdrawn)** The method of claim 17, 18, 19, or 20, wherein X is selected from the group consisting of Arginine, Lysine, Histidine, Phenylalanine, Tryptophan, Tyrosine, Leucine, Aspartate, Glutamate, Cysteine, Asparagine, Glutamine and Isoleucine.
22. **(Withdrawn)** The method of claim 19 or 20, wherein X is Methionine, Glycine or Valine.
23. **(Withdrawn)** The method of claim 17 or 18, wherein the reporter moiety is selected from the group consisting of: a transcription factor and a fluorescent marker.
24. **(Withdrawn)** The method of claim 17, 18, 19, or 20, wherein the translationally providing step is performed by a cell that expresses the ubiquitin-specific protease.
25. **(Withdrawn)** The method of claim 17, 18, 19, or 20, wherein the translationally providing step and the step wherein cleavage between Cub and X may occur is performed by a cell that expresses the ubiquitin-specific protease.
26. **(Withdrawn)** The method of claim 25, wherein the cell is a eukaryotic cell.
27. **(Withdrawn)** The method of claim 25, wherein the cell is a mammalian cell.
28. **(Withdrawn)** The method of claim 25, wherein the cell is a fungal cell.
29. **(Withdrawn)** The method of claim 25, wherein the cell is a plant cell.
30. **(Withdrawn)** The method of claim 25, wherein the cell is an insect cell.
31. **(Withdrawn)** The method of claim 25, wherein the cell is selected from the group consisting of: a human cell, a mouse cell, a rat cell, a hamster cell, a zebrafish cell, a Drosophila cell, a nematode cell, an *S. pombe* cell and an *S. cerevisiae* cell.
32. **(Withdrawn)** The method of claim 25, wherein the cell is selected from the group consisting of: an *A. thaliana* cell and an *N. tabacum* cell.

33. **(Withdrawn)** The method of claim 25, wherein the reporter moiety is a negative selectable marker, and the degree of activity of the reporter moiety is determined by incubating the cell under conditions that select against the negative selectable marker so that continued viability of the cell under negative selection conditions indicates that P1 binds P2.
34. **(Withdrawn)** The method of claim 33, wherein the negative selectable marker is selected from the group consisting of: URA3, Tk, codA, HygTk, Tkneo, TkBSD, PACTk, HygCoda, Codaneo, CodaBSD, PACCoda, GPT2, and HPRT.
35. **(Withdrawn)** The method of claim 33, wherein the negative selectable marker is selected from the group consisting of: TRP1, CYH2, CAN1.
36. **(Withdrawn)** The method of claim 25, wherein the reporter moiety is a positive selectable marker, and the presence or absence of the reporter moiety is determined by comparing the viability of the cell under conditions that select for the positive selectable marker to the viability of the cell under nonselective conditions, so that decreased viability of the cell grown under the positive selection conditions as compared to the viability of the cell grown under the nonselective conditions indicates that P1 binds P2.
37. **(Withdrawn)** The method of claim 36, wherein the positive selectable marker is selected from the group consisting of: URA3, Tk, Hygromycin resistance (Hyg<sup>R</sup>), neomycin resistance (neo<sup>R</sup>), puromycin resistance (PAC<sup>R</sup>) or Blasticidin S resistance (BlaS<sup>R</sup>), codA, HygTk, Tkneo, TkBSD, PACTk, HygCoda, Codaneo, CodaBSD, PACCoda, and GPT2.
38. **(Withdrawn)** The method of claim 36, wherein the positive selectable marker is selected from the group consisting of: HIS3, LYS2, LEU2, TRP2, ADE2.
39. **(Withdrawn)** The method of claim 17, 18, 19, or 20, wherein Nux contains at least one point mutation at amino acid 3 or amino acid 13 of a ubiquitin.
40. **(Withdrawn)** A method of determining whether a test compound agonizes or antagonizes the binding of two proteins to each other comprising the steps of:  
translationally providing a first fusion protein comprising segments P1, Cub-X, and RM, in an order wherein Cub-X is closer to the N-terminus of the first fusion protein



than RM, and a second fusion protein comprising segments Nux and P2, wherein P1 and P2 are proteins, which proteins may be the same or different, Nux is the amino-terminal subdomain of a wild-type ubiquitin or a reduced-associating mutant ubiquitin amino-terminal subdomain, Cub is the carboxy-terminal subdomain of a wild-type ubiquitin, X is an amino acid other than methionine and RM is an active reporter moiety; and,

comparing the amount of cleavage by a ubiquitin-specific protease between Cub and X by detecting the degree of the activity of RM in the presence of the compound with the amount of such cleavage that is expected in the absence of the test compound or in the presence of a standard compound, wherein increased cleavage indicates the test compound is an agonist and decreased cleavage indicates the test compound is an antagonist of P1/P2 binding.

41. **(Withdrawn)** A method of determining whether a test compound agonizes or antagonizes the binding of two proteins to each other comprising the steps of:

translationally providing a first fusion protein comprising segments P1, Cub-X, and RM, in an order wherein Cub-X is closer to the N-terminus of the first fusion protein than RM, and a second fusion protein comprising segments Nux and P2, wherein P1 and P2 are proteins, which proteins may be the same or different, Nux is the amino-terminal subdomain of a wild-type ubiquitin or a reduced-associating mutant ubiquitin amino-terminal subdomain, Cub is the carboxy-terminal subdomain of a wild-type ubiquitin, X is an amino acid and RM is an enzymatically active reporter moiety; and,

comparing the amount of cleavage by a ubiquitin-specific protease between Cub and X by detecting the degree of the enzymatic activity of RM in the presence of the compound with the amount of such cleavage that is expected in the absence of the test compound or in the presence of a standard compound, wherein increased cleavage indicates the test compound is an agonist and decreased cleavage indicates the test compound is an antagonist of P1/P2 binding.

42. **(Withdrawn)** A method for selecting an agonist or antagonist of P1/P2 binding from a library of test compounds, a multiplicity of said library compounds having no known agonist or antagonist activity for P1/P2 binding, comprising:
- 1) determining the agonist or antagonist activity of each test compound of the library according to the method of claim 40 or 41; and,
  - 2) selecting from the multiplicity at least one test compound that shows agonistic or antagonistic activity.
43. **(Withdrawn)** The method of claim 40 or 41, further comprising: selecting a candidate compound from a library of candidates which comprise 10 to 500 compounds, wherein multiple members of said library are not known to bind P1 or P2.
44. **(Withdrawn)** The method of claim 40 or 41, further comprising: selecting a candidate compound from a library of candidates which comprise 500 to 10,000 compounds, wherein multiple members of said library are not known to bind P1 or P2.
45. **(Withdrawn)** The method of claim 40 or 41, further comprising: selecting a candidate compound from a library of candidates which comprise greater than 10,000 compounds, wherein multiple members of said library are not known to bind P1 or P2.
46. **(Withdrawn)** The method of claim 42, wherein said library of candidate compounds is selected from the group: synthetic chemical library and natural chemical library.
47. **(Withdrawn)** The method of claim 40 or 41, wherein the candidate compound is a polypeptide.
48. **(Withdrawn)** The method of claim 47, wherein said polypeptide is supplied by a polypeptide library.
49. **(Withdrawn)** The method of claim 40 or 41, wherein the candidate compound is a small molecule compound.
50. **(Withdrawn)** The method of claim 40 or 41, wherein X is selected from the group consisting of: Arginine, Lysine, Histidine, Phenylalanine, Tryptophan, Tyrosine, Leucine, Aspartate, Glutamate, Cysteine, Asparagine, Glutamine and Isoleucine.
51. **(Withdrawn)** The method of claim 41, wherein X is Methionine, Glycine or Valine.

52. **(Withdrawn)** The method of claim 40 or 41, wherein the reporter moiety is a selectable marker.
53. **(Withdrawn)** The method of claim 52, wherein the selectable marker is selected from the group consisting of: URA3, HIS3, LYS2, HygTk, Tkneo, TkBSD, PACTk, HygCoda, Codaneo, CodaBSD, PACCoda, Tk, codA, GPT2, and HPRT.
54. **(Withdrawn)** The method of claim 52, wherein the selectable marker is selected from the group consisting of: TRP1, CYH2, CAN1.
55. **(Withdrawn)** The method of claim 40 or 41, wherein the reporter moiety is selected from the group consisting of: a transcription factor and a fluorescent marker.
56. **(Withdrawn)** The method of claims 40 or 41, wherein the translationally providing step is performed by a cell that expresses the ubiquitin-specific protease.
57. **(Withdrawn)** The method of claim 56, wherein the translationally providing step and the step wherein cleavage between Cub and X may occur is performed by a cell that expresses the ubiquitin-specific protease.
58. **(Withdrawn)** The method of claim 57, wherein the cell is a eukaryotic cell.
59. **(Withdrawn)** The method of claim 57, wherein the cell is a mammalian cell.
60. **(Withdrawn)** The method of claim 57, wherein the cell is a fungal cell.
61. **(Withdrawn)** The method of claim 57, wherein the cell is a plant cell.
62. **(Withdrawn)** The method of claim 57, wherein the cell is an insect cell.
63. **(Withdrawn)** The method of claim 57, wherein the cell is selected from the group consisting of: a human cell, a mouse cell, a rat cell, a hamster cell, a zebrafish cell, a Drosophila cell, a nematode cell, an S. pombe cell and an S. cerevisiae cell.
64. **(Withdrawn)** The method of claim 57, wherein the cell is selected from the group consisting of: an A. thaliana cell and an N. tabacum cell.
65. **(Withdrawn)** The method of claim 40 or 41, wherein Nux contains at least one point mutation at amino acid 3 or amino acid 13 of a ubiquitin.

66. **(Currently Amended)** A method of characterizing the sequence of a protein that binds a target protein comprising the steps of:

expressing a first and a second nucleic acid in a ubiquitin-specific protease expressing cell, which first nucleic acid encodes a target fusion protein comprising segments P1, Cub-X, and RM, in an order wherein Cub-X is closer to the N-terminus of the target fusion protein than RM and P1, wherein P1 is the target protein, Cub is the carboxy-terminal subdomain of a wild-type ubiquitin, X is an amino acid selected from the group consisting of arg, lys, phe, leu, trp, his, asp, asn, tyr, ile, glu, cys and gln, and RM is an enzymatically active reporter moiety, which second nucleic acid encodes a candidate fusion protein comprising segments P2 and Nux, wherein the second nucleic acid is a member of a library containing multiple different nucleic acids differing in the P2 segments they encode, P2 is a candidate segment and Nux is the amino-terminal subdomain of a wild-type ubiquitin or a reduced-associating mutant ubiquitin amino-terminal subdomain; recovering a clone of the cell expressing the first and second nucleic acid under conditions wherein a cell is selectable only in the absence of the enzymatic activity of RM; and, characterizing the second nucleic acid encoding P2.

67. **(Original)** The method of claim 66, wherein the enzymatically active reporter moiety is a negative selectable marker selected from the group consisting of: URA3, Tk, codA, HygTk, Tkneo, TkBSD, PACTk, HygCoda, Codaneo, CodaBSD, PACCoda, GPT2, and HPRT.
68. **(Original)** The method of claim 66, wherein the enzymatically active reporter moiety is a negative selectable marker selected from the group consisting of: TRP1, CAN1 and CYH2.
69. **(Currently Amended)** A method of characterizing the sequence of a protein that binds a target protein comprising the steps of:
- expressing a first and a second nucleic acid in a ubiquitin-specific protease expressing cell, which first nucleic acid encodes a target fusion protein comprising segments P1, Cub-X, and RM, in an order wherein Cub-X is closer to the N-terminus

of the target fusion protein than RM and P1, wherein P1 is the target protein, Cub is the carboxy-terminal subdomain of a wild-type ubiquitin, X is an amino acid selected from the group consisting of arg, lys, phe, leu, trp, his, asp, asn, tyr, ile, glu, cys and gln, and RM is an active reporter moiety, which second nucleic acid encodes a candidate fusion protein comprising segments P2 and Nux, wherein the second nucleic acid is a member of a library containing multiple different nucleic acids differing in the P2 segments they encode, P2 is a candidate segment and Nux is the amino-terminal subdomain of a wild-type ubiquitin or a reduced-associating mutant ubiquitin amino-terminal subdomain;

recovering a clone of the cell expressing the first and second nucleic acid under conditions wherein a cell is selectable only in the absence of an activity of RM; and, characterizing the second nucleic acid encoding P2.

70. **(Original)** The method of claim 69, wherein the active reporter moiety is selected from the group consisting of: a transcription factor and a fluorescent marker.
71. **(Original)** The method of claim 66 or 69, wherein the cell is a eukaryotic cell.
72. **(Original)** The method of claim 66 or 69, wherein the cell is a mammalian cell.
73. **(Original)** The method of claim 66 or 69, wherein the cell is a fungal cell.
74. **(Original)** The method of claim 66 or 69, wherein the cell is a plant cell.
75. **(Original)** The method of claim 66 or 69, wherein the cell is an insect cell.
76. **(Original)** The method of claim 66 or 69, wherein the cell is selected from the group consisting of: a human cell, a mouse cell, a rat cell, a hamster cell, a zebrafish cell, a Drosophila cell, a nematode cell, an S. pombe cell and an S. cerevisiae cell.
77. **(Original)** The method of claim 66 or 69, wherein the cell is selected from the group consisting of: an A. thaliana cell and an N. tabacum cell.
78. **(Original)** The method of claim 66 or 69, wherein the library of nucleic acids comprises 10 to 500 members, wherein fusions proteins encoded by multiple members of said library are not known to bind P1.

79. **(Original)** The method of claim 66 or 69, wherein the library of nucleic acids comprises 500 to 10,000 members, wherein fusions proteins encoded by multiple members of said library are not known to bind P1.
80. **(Original)** The method of claim 66 or 69, wherein the library of nucleic acids comprises greater than 10,000 members, wherein fusions proteins encoded by multiple members of said library are not known to bind P1.
81. **(Original)** The method of claim 66 or 69, wherein Nux contains at least one point mutation at amino acid 3 or amino acid 13 of a ubiquitin.
82. **(Currently Amended)** A kit for characterizing the sequence of a polypeptide that binds a target protein, which comprises:
- a first nucleic acid encoding a target fusion protein comprising a cloning site suitable for the insertion of a nucleic acid encoding a target protein sequence, segments Cub-X, and RM, in an order wherein Cub-X is closer to the N-terminus of the target fusion protein than RM and P1, wherein Cub is the carboxy-terminal subdomain of a wild-type ubiquitin, X is an amino acid selected from the group consisting of arg, lys, phe, leu, trp, his, asp, asn, tyr, ile, glu, cys and gln, and RM is an active reporter moiety, which activity allows for selection, whereby a fusion protein comprising the target protein sequence, Cub-X and RM can be expressed;
  - a second nucleic acid comprising an Nux segment encoding the amino-terminal subdomain of a wild-type ubiquitin or a reduced-associating mutant ubiquitin amino-terminal subdomain and a cloning site suitable for the insertion of a nucleic acid encoding a polypeptide sequence whereby a fusion protein comprising Nux and the polypeptide sequence can be expressed; and,
  - instructions indicating that a nucleic acid encoding a defined target protein sequence is to be inserted into the first nucleic acid and members of a library of nucleic acids encoding candidate polypeptides are to be inserted into the second nucleic acid, in order to characterize a polypeptide that binds to the target protein.
83. **(Original)** The kit of claim 82, wherein the active reporter moiety is a negative selectable marker selected from the group consisting of: URA3, Tk, codA, HygTk, Tkneo, TkBSD, PACTk, HygCoda, Codaneo, CodaBSD, PACCoda, GPT2, and HPRT.

84. **(Original)** The kit of claim 82, wherein the active reporter moiety is a negative selectable marker selected from the group consisting of: TRP1, CAN1, CYH2.
85. **(Original)** The kit of claim 82 wherein the active reporter moiety is selected from the group consisting of: a transcription factor and a fluorescent marker.
86. **(Original)** The kit of claim 82 wherein Nux contains at least one point mutation at amino acid 3 or amino acid 13 of a ubiquitin.
87. **(Original)** The kit of claim 82, wherein the expression of first and second nucleic acids are carried out in a cell.
88. **(Original)** The kit of claim 87, wherein the cell is a eukaryotic cell.
89. **(Original)** The kit of claim 87, wherein the cell is a mammalian cell.
90. **(Original)** The kit of claim 87, wherein the cell is a fungal cell.
91. **(Original)** The kit of claim 87, wherein the cell is a plant cell.
92. **(Original)** The kit of claim 87, wherein the cell is an insect cell.
93. **(Original)** The kit of claim 87, wherein the cell is selected from the group consisting of: a human cell, a mouse cell, a rat cell, a hamster cell, a zebrafish cell, a Drosophila cell, a nematode cell, an *S. pombe* cell and an *S. cerevisiae* cell.
94. **(Original)** The kit of claim 87, wherein the cell is selected from the group consisting of: an *A. thaliana* cell and an *N. tabacum* cell.
95. **(Original)** The kit of claim 82 wherein said instructions indicate that the library may comprise 10 to 500 members, wherein candidate polypeptides encoded by multiple members of said library are not known to bind said defined target protein.
96. **(Original)** The kit of claim 82 wherein said instructions indicate that the library may comprise 500 to 10,000 members, wherein candidate polypeptides encoded by multiple members of said library are not known to bind said defined target protein.
97. **(Original)** The kit of claim 82 wherein said instructions indicate that the library may comprise greater than 10,000 members, wherein candidate polypeptides encoded by multiple members of said library are not known to bind said defined target protein.

98. **(Currently Amended)** A kit for characterizing the sequence of a polypeptide that binds a target protein, which comprises:
- a first nucleic acid encoding a target fusion protein comprising a cloning site suitable for the insertion of a nucleic acid encoding a target protein sequence, segments Cub-X, and RM, in an order wherein Cub-X is closer to the N-terminus of the target fusion protein than RM and P1, wherein Cub is the carboxy-terminal subdomain of a wild-type ubiquitin, X is an amino acid selected from the group consisting of arg, lys, phe, leu, trp, his, asp, asn, tyr, ile, glu, cys and gln, and RM is an active reporter moiety, which activity allows for selection, whereby a fusion protein comprising the target protein sequence, Cub-X and RM can be expressed;
  - a library of second nucleic acids each comprising an Nux segment encoding the amino-terminal subdomain of a wild-type ubiquitin or a reduced-associating mutant ubiquitin amino-terminal subdomain and a nucleic acid encoding a polypeptide sequence, whereby a library of fusion proteins comprising Nux and the polypeptide sequences can be expressed.
99. **(Original)** The kit of claim 98, further comprising instructions indicating that a nucleic acid encoding a defined target protein sequence is to be inserted into the first nucleic acid, in order to characterize a polypeptide that binds to the target protein.
100. **(Original)** The kit of claim 98, wherein the active reporter moiety is a negative selectable marker selected from the group consisting of: URA3, Tk, codA, HygTk, Tkneo, TkBSD, PACTk, HygCoda, Codaneo, CodaBSD, PACCoda, GPT2, and HPRT.
101. **(Original)** The kit of claim 98, wherein the active reporter moiety is a negative selectable marker selected from the group consisting of: TRP1, CAN1, CYH2.
102. **(Original)** The kit of claim 98, wherein the active reporter moiety is selected from the group consisting of: a transcription factor and a fluorescent marker.
103. **(Original)** The kit of claim 98, wherein Nux contains at least one point mutation at amino acid 3 or amino acid 13 of a ubiquitin.
104. **(Original)** The kit of claim 98, wherein the expression of first and second nucleic acids are carried out in a cell.



105. **(Original)** The kit of claim 104, wherein the cell is a eukaryotic cell.
106. **(Original)** The kit of claim 104, wherein the cell is a mammalian cell.
107. **(Original)** The kit of claim 104, wherein the cell is a fungal cell.
108. **(Original)** The kit of claim 104, wherein the cell is a plant cell.
109. **(Original)** The kit of claim 104, wherein the cell is an insect cell.
110. **(Original)** The kit of claim 104, wherein the cell is selected from the group consisting of: a human cell, a mouse cell, a rat cell, a hamster cell, a zebrafish cell, a *Drosophila* cell, a nematode cell, an *S. pombe* cell and an *S. cerevisiae* cell.
111. **(Original)** The kit of claim 104, wherein the cell is selected from the group consisting of: an *A. thaliana* cell and an *N. tabacum* cell.
112. **(Original)** The kit of claim 98 wherein said library comprises 10 to 500 members, wherein candidate polypeptides encoded by multiple members of said library are not known to bind said defined target protein.
113. **(Original)** The kit of claim 98 wherein said library comprises 500 to 10,000 members, wherein candidate polypeptides encoded by multiple members of said library are not known to bind said defined target protein.
114. **(Original)** The kit of claim 98 wherein said library comprises greater than 10,000 members, wherein candidate polypeptides encoded by multiple members of said library are not known to bind said defined target protein.